

Claims

1. A method for isolating genes involved in the determination of a trait or phenotype of a plant species, said method comprising

- 5 a.) Identifying a set of nucleic acid sequences of genes, whose expression is correlated with a trait of interest.
- b.) creating a library of gene silencing constructs in a viral RNA vector, said viral RNA vector being capable of replication inside plant cells and said gene silencing constructs being targeted to the 10 nucleotide sequence of said nucleic acid sequences;
- c.) infecting a collection of individual plants of said plant species with said library of gene silencing constructs, whereby each plant is infected with at least one member of said library;
- d.) identifying a plant wherein said trait or phenotype is altered, using an assay adapted to said trait or phenotype;
- e.) isolating said gene involved in the determination of said trait or phenotype in said plant species, from said library based on the 15 nucleotide sequence to which said gene silencing construct in said identified plant was targeted.

20 2. The method of claim 1, wherein said viral RNA vector is capable of autonomous replication inside plant cells.

25 3. The method of claim 2, wherein said viral RNA vector is derived from cowpea mosaic virus.

30 4. The method of claim 1, wherein said viral RNA vector is capable of replication inside plant cells when the required factors are supplemented *in trans*.

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5. The method of claim 4, wherein said viral RNA vector is derived from a satellite RNA virus and said factors are supplemented by infection with a helper virus or helper virus RNA.

5 6. The method of claim 5, wherein said satellite RNA virus is satellite tobacco mosaic virus.

7. The method of claim 6, wherein said viral RNA vector comprises an origin of assembly of tobacco mosaic virus.

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8. The method of claim 7, wherein said helper virus is tobacco mosaic virus.

9. The method of claim 5, wherein said satellite RNA virus is satellite tobacco necrosis virus.

10. The method of claim 9, wherein said viral RNA vector comprises an origin of assembly of tobacco mosaic virus.

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11. The method of claim 10, wherein said helper virus is derived from tobacco necrosis virus and comprises a coat protein gene of tobacco mosaic virus.

12. The method of claim 11, wherein said satellite RNA virus is satellite tobacco necrosis vector strain 1 or 2 and said helper virus is derived from TNV-A.

13. The method of claim 11, wherein said satellite RNA virus is STNV-C and said helper virus is derived from TNV-D.

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14. The method of claim 1, wherein said gene-silencing constructs comprise antisense RNA.

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15. The method of claim 1, wherein said gene-silencing constructs comprise sense RNA.

5 16. The method of claim 1, wherein said gene-silencing constructs comprise an inverted repeat.

10 17. The method of claim 1, wherein said gene-silencing constructs comprise complementary stretch of at least 50 nucleotides of sense and antisense RNA.

15 18. The method of claim 17, wherein said gene-silencing constructs comprise complementary stretch of at least 100 nucleotides of sense and antisense RNA.

20 19. The method of claim 17, wherein said gene-silencing constructs comprise at least two copies of part of the nucleotide sequences of said collection of nucleic acids, said copies being in inverted repeat.

25 20. A method for the isolation of a nucleic acid with a specific function from a collection of nucleic acids, said collection of nucleic acids being characterized in that variation in the expression pattern of said nucleic acids is correlated with variation in a trait/phenotype of a plant harboring said nucleic acids, said method comprising the steps of

- a.) creating a library of gene silencing constructs in a viral RNA vector, said viral RNA vector being capable of replication inside, and said gene silencing constructs being targeted/adapted to the nucleotide sequence of said nucleic acids;
- b.) infecting a collection of plants with said library of gene silencing constructs, whereby each plant is infected with at least one member of said library;

c.) identifying plants with altered trait or phenotype using an assay adapted to said trait or phenotype.

21. The method of claim 20, further comprising the step of isolating said
5 nucleic acid with said specific function from said identified plant with altered
trait or phenotype.

22. A method for determining the function encoded by a nucleic acid
comprising a known nucleotide sequence in a plant, said method comprising

- 10 a.) providing a viral RNA vector, said viral RNA vector being derived from a satellite RNA virus, comprising a gene-silencing construct targeted to a gene comprising said known nucleotide sequence;
- 15 b.) infecting said plant with said viral RNA vector and a corresponding helper virus;
- c.) identifying an altered trait or phenotype of said co-infected plant.

23. A method for isolating essential genes in a plant, comprising

- 20 a.) creating a library of random gene-silencing constructs for said plant comprised within a viral RNA vector, said viral RNA vector being derived from a satellite RNA virus;
- b.) infecting a plant with at least one member of said library and with a corresponding helper virus; and
- c.) identifying plants developing a gene-silencing-construct-associated phenotype, preferably chlorosis or necrosis.

24. The method according to claim 23, further comprising the step of isolating the viral RNA vector from the tissue exhibiting the phenotype.

30 25. The method of claim 23, wherein said library is created by cloning random DNA fragments of said plant in a cDNA copy of the viral RNA vector.

26. The method of claim 23, wherein said library is created by cloning random cDNA fragments of said plant in a cDNA copy of the viral RNA vector.

27. The method of claim 23, wherein said library is created by cloning 5 random duplicated cDNA fragments in inverted repeat.

28. The method of claim 23, wherein said viral RNA vector is derived from STMV and comprises an origin of assembly of tobacco mosaic virus, and wherein said helper virus is tobacco mosaic virus.

10 29. The method of claim 23, wherein said viral RNA vector is derived from satellite tobacco necrosis virus and comprises an origin of assembly of tobacco mosaic virus and wherein said helper virus is derived from tobacco necrosis virus and comprises a coat protein gene of tobacco mosaic virus.

15 30. The method of claim 29, wherein said satellite RNA virus is satellite tobacco necrosis vector strain 1 or 2 and said helper virus is derived from TNV-A.

20 31. The method of claim 29, wherein said satellite RNA virus is STNV-C and said helper virus is derived from TNV-D.

25 *Sub C 1* 32. A method for the introduction of inhibitory RNA in the cytoplasm of plant cells, said method comprising:

- a.) introducing into said plant cell, a viral RNA vector comprising said inhibitory RNA or comprising a chimeric nucleic acid which when transcribed yields said inhibitory RNA, wherein said viral RNA vector is derived from a satellite RNA virus; and
- b.) introducing a corresponding helper virus into said plant cell.

30 33. The method of claim 32, wherein said inhibitory RNA comprises sense RNA.

- Sub C 2*
34. The method of claim 32, wherein said inhibitory RNA comprises antisense RNA.
- 5 35. The method of claim 32, wherein said inhibitory RNA comprises an inverted repeat.
- 10 36. The method of claim 32, wherein said inhibitory RNA comprises complementary stretch of at least 50 nucleotides of sense and antisense RNA.
- 15 37. The method of claim 36, wherein said inhibitory RNA comprises complementary stretch of at least 100 nucleotides of sense and antisense RNA.
- 20 38. The method of claim 32, wherein said viral RNA vector is derived from STMV and comprises an origin of assembly of tobacco mosaic virus, and wherein said helper virus is tobacco mosaic virus.
- 25 40. The method of claim 32, wherein said viral RNA vector is derived from satellite tobacco necrosis virus and comprises an origin of assembly of tobacco mosaic virus and wherein said helper virus is derived from tobacco necrosis virus and comprises a coat protein gene of tobacco mosaic.
- 30 41. The method of claim 39, wherein said satellite RNA virus is STNV-C and said helper virus is derived from TNV-D.

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Sub C 4

42. The method of any one of claims 32 to 41, wherein said plant is selected from *Nicotiana* spp., *Oryza sativa*, *Zea Mays*, *Brassica* spp., *Gossypium* spp., *Triticum* spp., *Arabidopsis* spp. or *Petunia* spp.

Sub C 5

43. A kit for introduction of inhibitory RNA in the cytoplasm of a plant cell, said kit comprising

- 10 a.) a viral RNA vector derived from a satellite RNA virus, said vector comprising a chimeric nucleic acid which when transcribed yields said inhibitory RNA or which comprises said inhibitory RNA; and
- b.) a corresponding helper virus.

Sub C 6

44. The kit of claim 43, wherein said inhibitory RNA comprises sense RNA.

Sub C 15

45. The kit of claim 43, wherein said inhibitory RNA comprises antisense RNA.

Sub C 16

46. The kit of claim 43, wherein said inhibitory RNA comprises an inverted repeat.

20 47. The kit of claim 43, wherein said inhibitory RNA comprises complementary stretch of at least 50 nucleotides of sense and antisense RNA.

- 25 48. The kit of claim 43, wherein said inhibitory RNA comprises complementary stretch of at least 100 nucleotides of sense and antisense RNA.

Sub C 30

49. The kit of claim 43, wherein said viral RNA vector is derived from STMV and comprises an origin of assembly of tobacco mosaic virus, and wherein said corresponding helper virus is tobacco mosaic virus.

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50. The kit of claim 43, wherein said viral RNA vector is derived from satellite tobacco necrosis virus and comprises an origin of assembly of tobacco mosaic virus and wherein said corresponding helper virus is derived from tobacco necrosis virus and comprises the coat protein gene of tobacco mosaic virus .

51. The kit of claim 50, wherein said satellite RNA virus is satellite tobacco necrosis vector strain 1 or 2 and said corresponding helper virus is derived from TNV-A.

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52. The kit of claim 50, wherein said satellite RNA virus is STNV-C and said corresponding helper virus is derived from TNV-D.

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